CRYO-COOLING and RADIATION DAMAGE

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Data quality pivotally affects the amount of biological information we obtain.

Farside copyrighted cartoon

Detailed biological information
The Plan:

• Cryo techniques
  – Optimising cryoprotection.
  – Testing at room temperature.
  – Storage and retrieval.
  – If nothing works...
Room temperature: HEWL crystal after 3 hours in a 2\textsuperscript{nd} generation synchrotron beam.
I24, Diamond, *in situ* data collection from a Bovine Enterovirus 2 crystal, room temperature, 0.5 s 20 μm x 20 μm beam


Beamline logo I24
(Gwyndaf Evans *et al.*)
Radiation Damage

Primary
Secondary

Protein: direct
Solvent: indirect

PRIMARY; inevitable, a fact of physics! Neutralise it?
SECONDARY, can we control it?
Haas and Rossmann 1970: lactate dehydrogenase
Acta Cryst B26, 998-1004.
ICE a major problem

Used wire loops

Also, a commercially available and easy to use cryostat (Cosier and Glazer 1986) made the technique accessible to many labs.

Radiation Damage

Significantly reduced at 100K: time factor of $\sim 70$

PRIMARY; inevitable, a fact of physics!
SECONDARY, can we control it?

Proportions?
All experiments reported in Acta Crystallographica. D, 1993 – Dec 2005

Year of deposition

Number of experiments

[Garman, in Protein Crystallisation (Ed. T.Bergfors) 2009]
Loop mounting is a MUCH gentler technique than capillary mounting. e.g. cyclin A, 5μm x 100μm x 300μm
Other advantages:

- Usually get a whole data set from a single crystal ⇒ higher QUALITY data. For MAD, the systematic errors are minimised by using only one crystal.
- Can harvest and store crystals while they are in peak condition.
- Small crystals and flat plates can be mounted easily.
- No secondary radiation damage during storage.
- New experiments are possible.
The Plan:

• Cryo techniques
  – **Optimising cryoprotection.**
  – Testing at room temperature.
  – Storage and retrieval.
  – If nothing works…
Cryocooling: HOW?

• Cool the crystal so fast that the water is vitrified and does not form crystalline ice. [Pure water: $\sim 10^{-5} \text{ s}$ for typical protein crystal sized drop]

• Add `antifreeze’ to increase time for cooling process. [1-2 s]

  i.e. `cryoprotect’

N.B. WE DO NOT WANT TO ‘FREEZE’ the crystal!

• Collect data at around 100K [below 130K and absolutely below 155K [Weik, 2001]]
• replace the water in mother liquor with cryo-agent, rather than diluting the mother liquor.
• test cryo-buffer alone in loop.

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Cryo-buffers.

- PEG < 4K → increase PEG, add small PEGs
- PEG ≥ 4K → add small PEGs
- 2/3rds of cases → add 15 - 25% glycerol
- MPD → harvesting buffer, increase MPD concentration.
- Salt → add MPD and/or ethylene glycol or glycerol
  → increase conc/add salt. Lithium salts good.
  → Exchange salt. e.g. 100% 8M Na Formate.

N.B. Low salt needs > concentration of cryoprotectant than high salt.
Sugars, paratone N, combinations, +++
Acupuncture needles:
[free samples available from me]

- No loss of liquid
- Slightly flexible
- Fine
- Different sizes available.

CRYSTAL MANIPULATION:

- Skin on protein drop:
  - Gentle surgery
  - Skin doesn’t diffract: just increases background

- Cats’ whiskers, horse eye lashes, horse tail hairs, etc etc
Manipulation of crystal from growth drop. A cryo-loop, or a syringe with flexible hose give much better control than a Gilson.
Ellie
2002-2009

Megan, 1988-present
Illegible table of minimum concentrations of glycerol required to cryoprotect Hampton Screen I. For emergency use only (done by dilution)

Does NOT mean that glycerol is best!!
Transfer of crystal into cryobuffer:

1) Dialysis of cryoprotectant.

2) Co-crystallisation with cryoprotectant agent:
   glycerol is already known to help in some cases

3) Rapid transfer
   – Straight into final concentration for up to 5 mins
   – can just `sweep through’- 0.5 sec
   – Sequential soaks in increasing concentrations.

WANT TO MINIMISE HANDLING, as handling can increase the mosaic spread. i.e. 2) is BEST:…..

SOLUBILITY versus OSMOTIC shock
Crystal transfer optimisation:
• balance osmotic shock and attack of crystal surface
• serial transfers: minimise handling and dehydration
Hardware development: standardisation...
Litho-loops, etched mylar, ActiLoop polymer
Molecular Dimensions

Micro-mounts, microfabricated polyimide film. MiTeGen
[Thorne et al (2003), JAPC 36, 1455.]
Fishing:

- minimise handling
- minimise liquid round crystal
- fish near cryogen
- loop perpendicular to liquid

N.B. Acupuncture needles. Salt crystals in loop.
Flash cool into cryogen:

• Straight into liquid nitrogen: have a standard pin length AND blow/flap away surface cold nitrogen from FULL Dewar.

• Stream cool into nitrogen gas stream held at around 100K: pre-align pin. have a standard pin length!

PRACTICE!!!
(but not with your most precious crystals)
Humidity device to avoid dehydration during mounting

Especially effective for those mother liquors which phase separate when crystallisation drop is opened.

100K nitrogen stream

Dry air or \( \text{N}_2 \)

Dry air or \( \text{N}_2 \)

Crystal in fibre loop

X-ray beam

Figure 1

- Hollow metal pin
- Pin holding screw
- Metal top hat
- Fixing screw
- Goniometer head
- Metal locating pin
- Magnetic rubber disc
- Metal support for magnetic disc

Flat on side for self-opposing tweezers

Lateral vent
• Pre-centre loop.
• Speed of transfer from drop
• Block stream [avoid dehydration]
• Speed of cooling
Why bother to cover the nitrogen stream?

- Avoid crystal being dehydrated by dry nitrogen/air.
- You may wave crystal in and out of stream while cooling it: slow cooling will give ice.
- Want to cool it FAST. Much easier to do that if you whip the cover away once crystal is in position.
DIFFRACTION?

- NO …

**Does the crystal diffract at room temperature??**

- NO
  - Back to crystallisation trials

- YES
  - Change cryo-protocol trials

- YES… then take data and/or store it in a Dewar.
The Plan:

• Cryo techniques
  – Optimising cryoprotection.
  – **Testing at room temperature.**
  – Storage and retrieval.
  – If nothing works…
(Much) easier RT mounting method

Allows protein crystals to be mounted at room temperature in loop.

Capillary prevents drop drying out at room temperature.

Also eliminates crystal manipulation between room temperature and cryo-cooled datasets.

Room temperature crystal mounting methods

Kalinin et al. (2005).
MOSAICITY:

• Want to minimise mosaicity but it often increases.
• Are cryoconditions optimised?
  - solutions
  - transfer
• Speed of transfer to cryogen
• Speed of cooling
• Size of crystal:

Should be able to reproduce room temp mosaicity [need to know it!].
Minimise mosaic spread to optimise data quality.

Should be able to reproduce room temp mosaicity [need to know it!].
Cryo-protocol Optimisation: maximising resolution, [minimise mosaicity]

Fig. 2. Variation of mosaicity and diffraction limit of GPb crystals with percentage of glycerol in the buffer. The error bars represent statistical counting errors only.

The Plan:

- Cryo techniques
  - Why cool? Radiation damage.
  - Optimising cryoprotection.
  - Testing at room temperature.
  - **Storage and retrieval**
  - If nothing works...
Crystal storage and retrieval

Farside copyrighted cartoon
Hyperquenching: want a FAST cool

Keep Dewar full or remove gas layer from above liquid.

STORAGE:

• Label the vials beforehand.
• Label the canes.
• Allow transport Dewars to dry out after each trip.
Dewar drying rack, Dept Biochemistry, Oxford, UK
Excellent Dewar testing protocol

http://smb.slac.stanford.edu/facilities/hardware/cryotoools/shipping-dewar-testing.html

GOOGLE search (first hit):

ssrl dewar testing

Change storage LN$_2$ every 3 months.
Magnetic wand
Vial holder
Crystal Handling under LN2

- Use appropriate tools
- Have a small working Dewar: change the LN2 in it often.
- Wait for LN2 to stop bubbling before trying any manipulations.
- Only move one hand at a time
- Steady the stationary hand on edge of Dewar

Step by step instructions:
http://www.oxcryo.com/about/lmb-guide/
SAFETY: gloves and goggles

What am I doing wrong?
Absolutely NO ice of ANY sort.
CRYSTAL SOAKS:

• Use same cooling protocol
  [soak time, solution concentrations]
  BEST to use same human being too!

• MIR/substrate- put it in the cryo-buffer too:
  N.B. competitive inhibition with cryo-agent.

• Non-isomorphism: MR to native first
  [Crick and Magdoff 1956]
Bound cryoprotectant agent.

The Plan:

• Cryo techniques
  – Optimising cryoprotection.
  – Testing at room temperature.
  – Storage and retrieval.
  – **If nothing seems to work....**
If nothing seems to work:

- Diffraction at room temperature?
- Cryo-solutions
- Transfer/handling/soaking – vary time and temperature [e.g. try 4º overnight]
- Cryogen choice: nitrogen gas/liquid
- Osmolarity matching
- Crystal annealing
- Swap buffer
- Try more than once.
Theoretical study: starts to rationalise experimental practice.

Heat transfer study by a proper mechanical engineer!

Most to least important factors:
1) Crystal solvent content and solvent composition.
2) Crystal size and shape.
3) Amount of residual liquid around the crystal
4) Cooling method (liquid plunge \textit{versus} gas stream).
5) Choice of gas/liquid.
6) Relative speed between cooling fluid and crystal.

[S. Kriminski, M. Kazmierczak and R.E. Thorne
If nothing seems to work:

- Diffraction at room temperature?
- Cryo-solutions
- Transfer/handling/soaking – vary time and temperature [try 4º overnight]
- Cryogen choice
  - Osmolarity matching
  - Crystal annealing
- Swap buffer
- Try more than once.
Osmolarity matching:

1) Look up osmotic pressure of mother liquor in CRC Handbook of Physics and Chemistry, Section D-232. 11th column is O: Os/kg

2) Look up o.p. of cryoprotectant agent.

3) Modify conc. of mother liquor to minimise change in osmotic pressure.

Osmotic shock: compresses crystal $\Rightarrow$ cracks $\Rightarrow$ Mosaic spread increases $\Rightarrow$ resolution lower.

`Quick dips’ give greatest osmotic shock but minimise time for cryoprotectant attack.
## 23 GLYCEROL, CH₃OHCHOHCH₂OH

**Molecular Weight** = 92.09
**Relative Specific Refractivity** = 1.109

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## 24 HYDROCHLORIC ACID, HCl

For Values of 0
Osmotic Pressure Matching:
Example

Osmality: Os/kg

- 2.0M NaCl
  50mM pH 7.8 Tris HCl \[3.95\]
- Need 20% glycerol \[2.9\]

From Tables, 0.55M NaCl has o.p. 1.05 Os/kg

⇒ Try 0.55 M NaCl, 20% glycerol

50mM pH 7.8 Tris HCl
Crystal annealing: remove ice, reduce mosaicity and increase resolution

- Block stream temporarily (1-10 secs) OR
- Put crystal back in cryo-buffer solution.
- Then flash cool again.
- Worth a try (can repeat several times).
- Works sometimes.

[misnomer: slow heat and fast cool is really `tempering']

Hanson et al. (2003) Meth Enzym 368, 217
Understanding why annealing sometimes works.

Cryoprotectant agent concentration: match contraction of lattice to contraction of bulk solvent to avoid lattice distortion, higher mosaicity etc.

Not enough cryoprotectant
Water exported

Too much cryoprotectant
Water imported

If nothing seems to work:

- Diffraction at room temperature?
- Cryo-solutions
- Transfer/handling/soaking – vary time and temperature [4°]
- Cryogen choice
- Osmolarity matching
- Crystal annealing
- Swap buffer
- Try more than once.
Have at least 6 goes…
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<th>Advantages</th>
<th>Disadvantages</th>
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<td>• Reduced radiation damage.</td>
<td>• Expensive equipment</td>
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<tr>
<td>• Gentler mounting</td>
<td>• Increase in mosaic spread.</td>
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<tr>
<td>• Lower background</td>
<td>• Need to invest time.</td>
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</tr>
<tr>
<td>• Fewer crystals</td>
<td></td>
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<tr>
<td>• Can ship crystals</td>
<td></td>
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<tr>
<td>• Use crystals when ready.</td>
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</table>
Cryocrystallography: for more gory details see:

The Crystallographer’s DILEMMA:

Rate of damage versus diffraction intensity

Still the first day and my brain is already over full…
Radiation damage:
The Plan:

• What are the symptoms?
• What is it?
• Why do we care? Effect on MAD/SAD.
• How do we calculate the Dose?
• What do we know/would like to know?
Intensity decrease

Loss of diffraction

Incomplete data from crystals

RT
SSRL, 19 hours, 9.1, 1998
BRIGHTNESS OF X RAYS has increased by many orders of magnitude since the advent of synchrotron-radiation sources. Undulators in storage rings are the brightest source.
Iron containing protein, ESRF

1995 onwards: 100 K

BUT THEN, 1999:

2002: 30µm beam

[Tassos Perrakis]
Also observe spectral changes

Dataset 1

Happens during 1 dataset at 100K for some crystals

Unit cell volume expansion

Increase in Wilson B factors

Intensity decrease

Loss of diffraction

Incomplete data from crystals

Dataset 10

$1 \times 10^{12}$ ph s$^{-1}$ into 100$\mu$m square slits

$3 \times 10^{13}$ ph s$^{-1}$ into 50$\mu$m $\times$ 70$\mu$m $[10^{14}]$

‘GLOBAL’ damage

$10^{12}$ ph s$^{-1}$ into 100$\mu$m square slits

$3 \times 10^{13}$ ph s$^{-1}$ into 50$\mu$m $\times$ 70$\mu$m $[10^{14}]$
Intensity Decay at 100K

Normalised Intensity vs Dose: apoferritin

![Graph showing mean intensity of reflections vs dose for apoferritin with three different apoproteins: Apo1 (half-life \(D_{1/2} = 4.7 \times 10^7\) Gy), Apo2 (half-life \(D_{1/2} = 5.1 \times 10^7\) Gy), and Apo3 (half-life \(D_{1/2} = 4.6 \times 10^7\) Gy).]
Unit cell volume increase

Y = A + B * X
A = 0.01154 +/- 0.02658
B = 1.09977E-7 +/- 7.05011E-9
R = 0.98006
Data Parameters affected by Radiation Damage

- $I/\sigma(I)$ or resolution limit
- $R_{\text{merge}}$
- Scaling B factors
- Mosaicity
- Unit Cell expansion
  - a) function of dose
  - b) function of cryogen temperature

Could this be an on-line damage metric?


No!

What global damage metric should we use and against what should we plot it?

- $\frac{I_n}{I_o}$
- Not $\frac{I}{\sigma(I)}$
- B factors?
- An $R_{\text{meas}}$ type measure?
Dose estimation (the $x$-axis!)

$$\text{Dose} = \frac{\text{energy absorbed}}{\text{unit mass}}$$

$$= \frac{J}{\text{kg}} = \text{Gy}$$
DOSE

• DOSE is the ENERGY lost per KILOGRAMME (!!)

• Measured in Joules/kg i.e. the absorbed energy per unit mass.

• Fundamental metric against which to measure damage.

• FLUX is in photons/second.

• Flux density is in photons/second/unit area.

• Dose takes care of the physics but NOT the chemistry.
Can define (to plot against dose):

- Coefficient of sensitivity $\alpha$ change in relative isotropic B factor:

$$s_{AD} = \frac{\Delta B_{rel}}{8\pi^2 \Delta D} \quad (\text{e.g. HEWL@100 K} = 0.012 \text{ Å}^2/\text{Gy})$$

Global damage: summary

- Incomplete data.
- Causes non-isomorphism: MAD/SAD problems.
- No significant ($< \times 2$) dose rate effect at 100 K at current flux densities ($10^{15}$ ph/s/mm$^2$).
- No significant ($< \times 2$) temperature dependence, but weak minimum at around 50 K.
- Damage to lattice due to hydrogen abstraction and then build up?
- Heating not significant at current flux densities.
- For a particular system is predictable/can be modelled (using a sacrificial crystal)
Changes upon X-ray exposure

Relative intensity loss

Increased unit cell volume

Increased relative scaling B values

Electron density maps

- Loss of summed intensity
- Increased unit cell volume
- Increased B values
- Loss of electron density

B values Å\(^2\) (0=blue, 70 =red)

Ds1  Ds10
Specific structural damage observed:
- Disulphide bridges broken
- Decarboxylation of glutamate and aspartate residues
- Tyrosine residues lose their hydroxyl group
- Methionines: carbon-sulphur bond cleaved


- Rupture of covalent bonds to heavier atoms: C-Br, C-I, S-Hg

Note that if this were due to primary damage alone, damage would be in order of absorption cross sections of atoms, which it is not.
Specific Damage: summary.

- Can compromise biologically relevant observations (e.g. damage enzymatically important glutamates).
- Metallo-enzymes are reduced by X-ray beam.
- Perhaps weakly dose rate dependent (< × 2)
- Perhaps weakly wavelength dependent (< × 2)
- Weakly temperature dependent (varying results) (< × 2)
- Can be reduced with certain scavengers: but very conflicting results (mainly < × 2, benzoquinone RT × 9)
- We DON’T understand pecking order of damage within an amino acid group pH? Solvent accessibility? Neighbouring amino acids?
Radiation damage:
The Plan:

• What are the symptoms?
• **What is it?**
• Why do we care? Effect on MAD/SAD.
• How do we calculate the Dose?
• What do we know/would like to know?
PHYSICS of the interaction of X-rays with crystals.

A) Diffraction

B) Absorption = Energy loss

N.B. > 90% of the beam does not interact at all, but goes straight through.
A) Primary X-ray interaction processes with crystal and solvent.

Thomson (Rayleigh, coherent) scattering

[8% at 1Å]

ELASTIC - no energy loss.
Primary X-ray interaction processes with crystal and solvent.

Thomson (Rayleigh, coherent) scattering

ELASTIC - no energy loss.

[8% at 1Å]
Primary X-ray interaction processes with crystal and solvent.

Thomson (Rayleigh, coherent) scattering

ELASTIC - no energy loss.
   Coherent – adds vectorially and gives diffraction pattern.
   Small proportion of total scattering: 8% at 1Å

BUT IT IS THE BIT WE WANT!!
Compton (incoherent) scattering

X-ray transfers some energy to atomic electron and thus has lower energy (higher wavelength).
Compton (incoherent) scattering

X-ray transfers some energy to atomic electron and thus has lower energy (higher wavelength).
Incoherent – part of X-ray background in images.
Also a small proportion of total scattering: 8% at 1Å
Photoelectric Absorption

84% at 1Å

INELASTIC.
Photoelectric Absorption

INELASTIC.
X-ray transfers all its energy to an atomic electron, which is then ejected. Each 12 keV primary photoelectron can give rise to up to 500 ionisation events. Atom can then emit a characteristic X-ray or an Auger electron to return to its ground state.

\[
\sigma_{\text{tot}} = \sigma_{\text{pe}} + \sigma_{\text{inc}} + \sigma_{\text{coh}}
\]

84% + 8% + 8% at 1Å
Photoelectric Cross Sections (barns/atom) at 13.1 keV

\[ 1 \text{ barn} = 10^{-28} \text{m}^2 \]

A few heavy atoms can make a big difference.

[Ravelli et al., JSR, (2005) 12]
Photoelectric Cross Sections (barns/atom) at 13.1 keV

A few heavy atoms can make a big difference.

[1 barn = $10^{-28}m^2$]

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Photoelectric Cross Sections (barns/atom) at 13.1 keV

\[1 \text{ barn} = 10^{-28} \text{m}^2\]

A few heavy atoms can make a big difference.

[H C N O S]
Photoelectric Cross Sections (barns/atom) at 13.1 keV

\[
[1 \text{ barn}=10^{-28} \text{m}^2]
\]

A few heavy atoms can make a big difference.

[H, C, N, O, S]

[Se]

[Ravelli et al., JSR,(2005) 12]
Beam absorption ($\lambda=1\text{Å}$) by a protein crystal

Native HEWL 100 µm thick

Platinum derivatised (1/molecule) HEWL 100 µm thick

N.B. INCIDENT FLUX is the SAME but the absorbed dose is DOUBLE
A few heavy atoms in the solvent can make a BIG difference to the absorption cross section and this the dose rate for the SAME flux.

e.g. Cacodylate buffer (arsenic, mass 75 cf selenium = 79)

**BACK SOAKING to REMOVE**

Non-specifically bound heavy atoms

e.g. a brominated DNA-protein complex will radiation damage much faster than a native crystal, and will de-brominate during data collection [Ennfar et al, Acta Cryst D (2002) 1263-1268].
Radiation damage:
The Plan:

• What are the symptoms?
• What is it?
• Why do we care? Effect on MAD/SAD.
• How do we calculate the Dose?
• What do we know/would like to know?
Effect on MAD/SAD.

- Failure of structure determination (Multi-wavelength anomalous dispersion MAD) due to creeping non-isomorphism –
  a) cell expansion and
  b) movement of molecule in unit cell
  c) structural changes DURING experiment.
Non-isomorphism: DISASTER!

Crick and Magdoff (1956) showed that for a 0.5% change in all three unit cell dimensions of 100Å, the intensity would change by

15% at 3Å

for general reflections

[Crick and Magdoff (1956) Acta Cryst 9, 901-908]

i.e. MAD/SAD phasing signals (<5%) washed out completely.
Radiation damage:
The Plan:

• What are the symptoms?
• Why do we care? Effect on MAD/SAD.
• What is it?
• How do we calculate the Dose?
• What do we know/would like to know?
DOSE Postulate:

- There is a MAXIMUM dose (Joules/kg = Gy) which protein crystals can tolerate which depends only on the PHYSICS of the situation.

- Crystal might not reach that limit due to chemical factors, but it will not last BEYOND the limit.

- Need to be able to calculate the DOSE: RADDOSSE

V1: Murray, Garman & Ravelli (2004) JAPC, 37, 513-522
V3: Paithankar & Garman (2010), Acta D 66, 381-388
Typical MX experiment

A dose of 1 MGy will be absorbed in 10 s by a 100 μm cubed metal free crystal in a 100 μm x 100 μm 12.4 keV (1 Å) X-ray beam flux $10^{12}$ photons s$^{-1}$

MX at 100 K: for above example, The 30 MGy dose ‘limit’ is reached in approx 5 mins
Dose calculation

To find the energy deposited per unit mass in the crystal, need to characterise two things:

The Beam

The crystal
Number of Amino Acids

‘HA’ atoms per monomer, e.g. S, Se, Hg

Solvent - concentrations of components, e.g. Na\(^+\), Cl\(^-\)
Calculating Dose (RADDOSE)

Crystal Characteristics

- No of molecules per unit cell
- Solvent content
- No residues
- Crystal size

Beam Characteristics

- Size and profile
- Exposure time
- Flux

Calculation of absorption coefficients

5C + 1.35N + 1.5O + 8H

absorption coefficients

- e.g. apoferritin: 0.406mm⁻¹
- holoferritin: 1.133mm⁻¹
The heavy atom ($z \geq 16$) content of a crystal is not crystallographically defined.

N.B. INCIDENT FLUX is the SAME but the absorbed dose is DOUBLE

\[ \lambda = 1 \text{Å} \]
\[ 100 \, \mu \text{m thick xtal} \]
Quantification at cryotemperature

- Holoferritin and Apoferritin as model
  - Absorption coefficient differs by factor of 2

- Linear dependence on dose

- $D_{1/2} = 4.3 \times 10^7$ Gy
  Where $D_{1/2}$ is dose to half the intensity lost
Experimental Dose Limit

\[ I_0 \times 1/2 \]

\[ D_{1/2} = 4.3 (\pm 0.4) \times 10^7 \text{ Gy} = 43 \text{ MGy} \]

Henderson limit:

\[ D_{1/2} = 2.0 \times 10^7 \text{ Gy} = 20 \text{ MGy} \]

Howells et al (2005) recommended a resolution dependent limit of 10 Gy/ Å
Radiation damage to protein crystals: www.raddo.se

- Fluorescence yield
- Crystal characteristics
- Beam characteristics (pin diode)
- Intensity decay
- Unit cell
- Electron density
- B values
- Assessing damage
- RADDOS-3D
- Counts → Photons → Dose

An experimental dose limit for protein crystallography of $3.0 \times 10^7$ Gy
Experimental Dose Limit (100K)

For $I_0 \times 1/2$

$D_{1/2} = 4.3 \pm 0.4 \times 10^7$ Gy = 43 MGy

BUT

Suggested limit to retain biological ‘fidelity’

$I_0 \times 0.7$

$D_{0.7} = 3.0 \times 10^7$ Gy = 30 MGy

$D_{0.7}$ for ferritin corresponds to 107 photons/unit cell

Robin Leslie Owen, Enrique Rudiño-Piñera, Elspeth F. Garman.
Radiation damage:  
The Plan:  

- What are the symptoms?  
- Why do we care? Effect on MAD/SAD.  
- What is it?  
- How do we calculate the Dose?  
- **What do we know/would like to know?**
attenuators or beam defocussing

X-rays:
- flux, wavelength
- dose, dose rate
- beam size cf crystal size

Crystal in loop:
- heavy atom content (Se, S etc), solvent content,
- solvent composition, crystal size and S/V,
- amount of residual liquid around crystal,
- choice and concentration of cryoprotectant agent,
- time in cryobuffer, flash-cooling method (stream or liquid),
- cryogen used to flash-cool, amount of crystal manipulation,
- humidity, speed of experimenter when flash-cooling.

[Garman, Current Opinion of Structural Biology 2003, 9, 545-551]
PROBLEM: how do we know that we are making any difference?

• In order to investigate the effects of various parameters on the radiation damage process, we need a robust radiation damage METRIC which is preferably ON-LINE during the diffraction experiment. No unanimous metric currently/ results from different metrics do not agree.

• Structural changes occur before degradation of diffraction quality is obvious.
Work so far / ongoing:

- Lower the wavelength? Lots of anecdote + now some systematic results: *no effect on damage rate.*
- Unit cell expansion as a metric? *No!*
- Change/ regulate the dose/dose rate regime? *No, not at current!*

- Effect on MAD/SAD? Order of data collection?
- Minimum crystal size? Several papers. *(see Holton 2009)*
- Beam heating. *Not a big factor at current flux densities.*
Work so far / ongoing:

- X-ray absorption – important parameters defined.
- Remove oxygen? Nothing yet.
- Radiation damage Induced Phasing (RIP)
- Software developments – big progress.
- Add radical scavengers: results disagree.
- Biological implications/applications to mechanistic studies. Now many.
- Room temperature studies: dose rate effects? Results disagree.

N.B. Need for systematic statistically significant experiments.

Series of Radiation Damage Workshops
RD2: Dec 2001

RD3: Nov 2003

JSR, Nov 2002, 8 papers

JSR, May 2005, 9 papers
RD4: March 2006

Journal of Synchrotron Radiation
Editors: Å. Kvick, D. M. Mills and T. Ohta

Radiation Damage in Macromolecules

Guest Editors: Elspeth Garman and Sean McSweeney

journals.iucr.org
International Union of Crystallography
Blackwell Munksgaard

JSR, Jan 2007, 14 papers

RD5 March 2008

Journal of Synchrotron Radiation
Editors: G. E. Ice, Å. Kvick and T. Ohta

Radiation Damage in Macromolecular Crystallography

Guest Editors: Elspeth Garman and Colin Nave

journals.iucr.org
International Union of Crystallography
Wiley-Blackwell

JSR, March 2009, 8 papers
RD8: April 2014

RD9: 9-11 March 2016
MAX IV Lund

More JSR papers in 2017 from RD9??

General summary in:

Also a Beginner’s guide to RD in Holton (2009) JSR 16,133-142

JSR, March 2015, 8 papers
Summary 1: what can YOU, the experimenter do?

- Do not be afraid to merge data taken from different isomorphous crystals which all had lower doses.

- Back soak non-specifically bound heavier atoms out of your crystals.

- Be ‘absorption aware’ of the contents of your crystal (e.g. Se and buffer) and if possible, avoid cacodylate buffer (arsenic mass=75).

- Match beam size to crystal size
Summary 2: what can YOU, the experimenter do?

- Scavengers: try electron scavengers at 100 K (nitrate/ascorbate/benzoquinone).

- Dose ‘spreading’: use a tophat profile beam if possible. Consider Helical/Translational data collection.

- So you can estimate the dose, ASK at the beamline:
  - What is the flux today at this energy and with this slit size (‘flux density’)?
  - What is the beam profile today at this beam energy? FWHM in $x$ and $y$?
Current status: radiation damage in protein crystals

• Understand a lot more than twelve years ago, but still not nearly enough…
• Understand how to do experiments better.
• Research has prompted some very exciting new approaches.
• Many complementary methods now being used on the problem in concert with crystallography
• Experiments must involve more than one sample (!) to get statistically significant results: labour intensive and time consuming. Also MUST know incident FLUX density…

• **Radiation damage has DEFINITELY become a mainstream concern**
Still the first day and my brain is already over full…
The Crystallographer’s DILEMMA:

Rate of damage versus diffraction intensity